

**Figure 1**

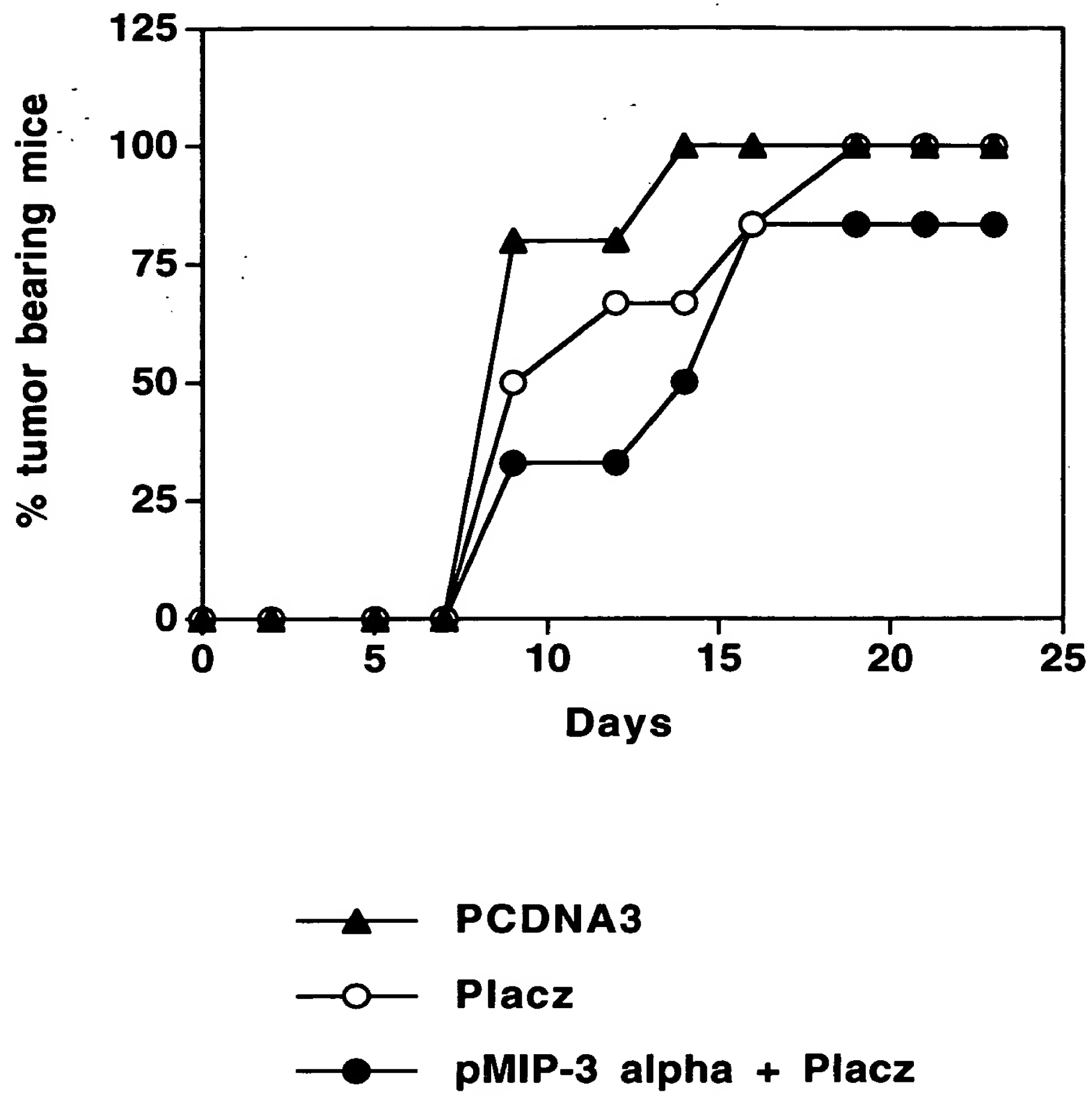
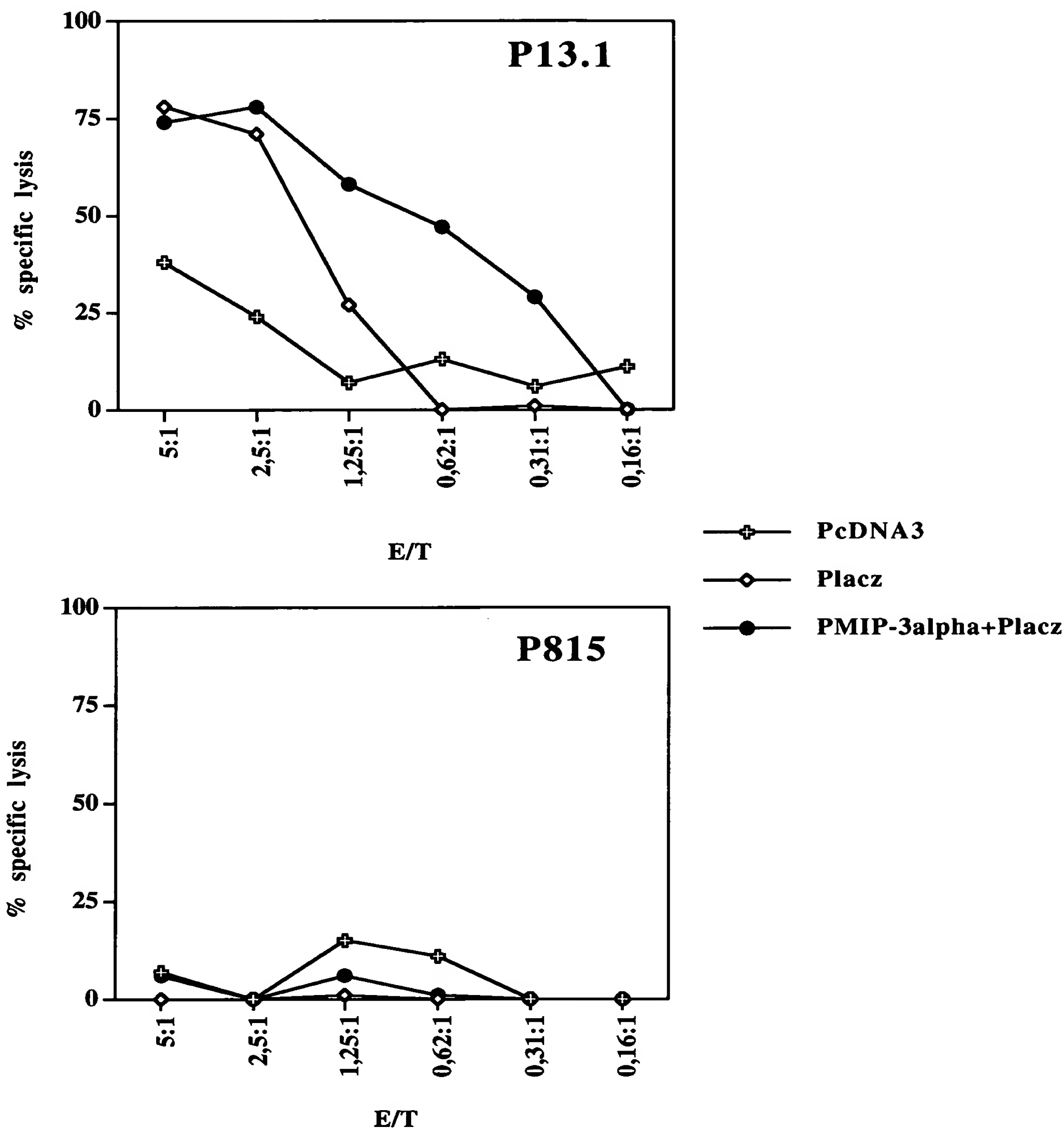


Figure 2



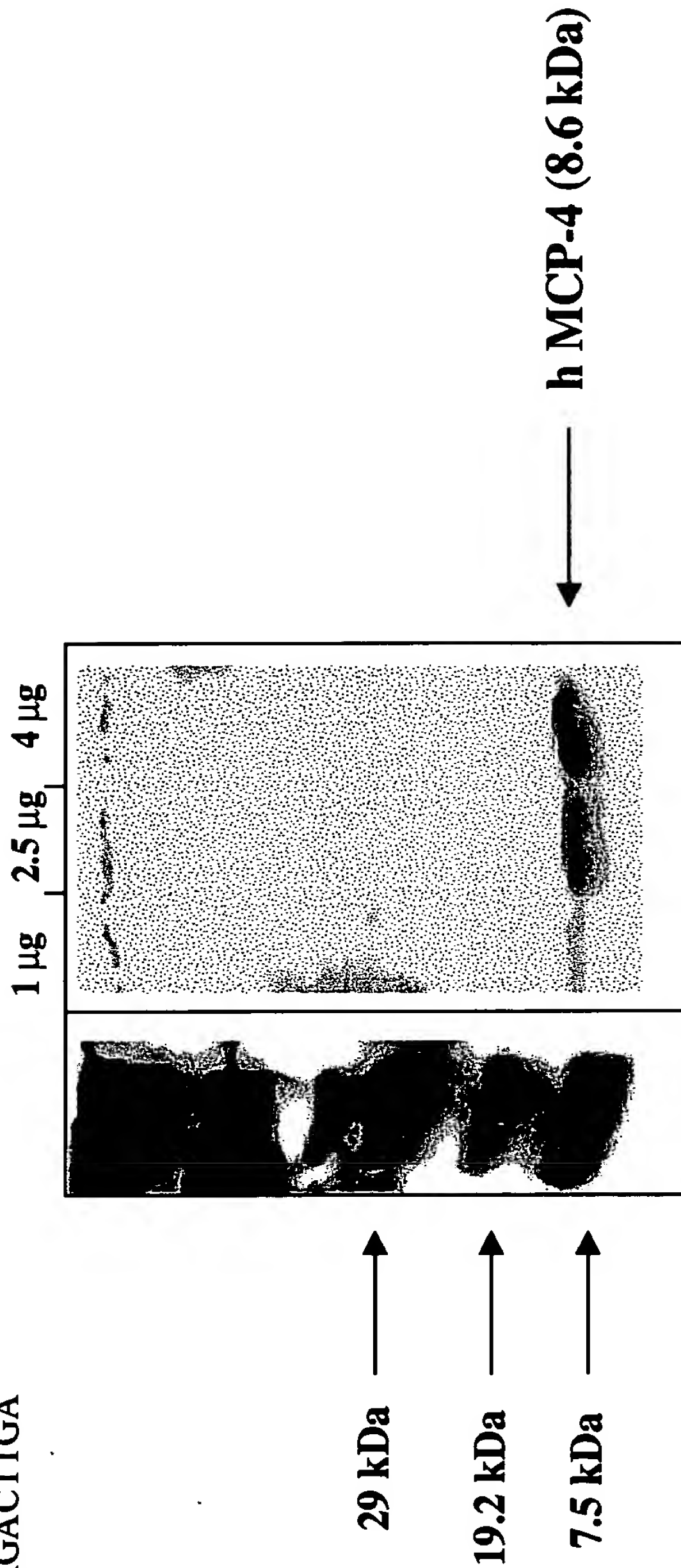
## hMCP-4 chemokine

- Nucleotide sequence (coding only)

ATGAAAGTCTCTGCAGTGCTTCTGTGCCT  
GCTGCTCATGACAGCAGCTTTCAACCCCC  
AGGGACTTGCTCAGCCAGATGCACCTCAA  
CGTCCCATCTACTTGCTGCTTCACATTTA  
GCAGTAAGAAAGATCTCCTTGCAGAGGCT  
GAAGAGCTATGTGATCACCAACAGCAGG  
TGTCCTCCAGAAAGGCTGTCTCTTCAGAAC  
CAAACTGGGCAAGGAGATCTGTGTGAC  
CCAAAGGAGAAAGTGGGTCCAGAAATTATA  
TGAAACACCTGGGCCGGAAAGCTCACAC  
CCTGAAGACTTGA

- Amino acid sequence (leader sequence not present in recombinant protein in italics)

*MKVSAVLLCLLLMTAAFN**PQGLAQPDALNV*  
*PSTCCFTFSSKKISLQRLKSYVITTSRCPQK*  
*AVFRTKL**GKEICADPKEK* *WVQNYMKHL*  
*GRKAHTLKT*



SDS-PAGE (18%) and silver staining of human recombinant MCP-4

Figure 3

(A) Local recruitment of CD11b+ cells 2 h following hMCP-4 injection  
(B) Increase of dendritic cells in the draining lymph node 20 hours after hMCP-4 s.c. injection: absolute numbers. Right panel statistical difference between hMCP-4 and controls  $p < 0.01$  (Student's t test)

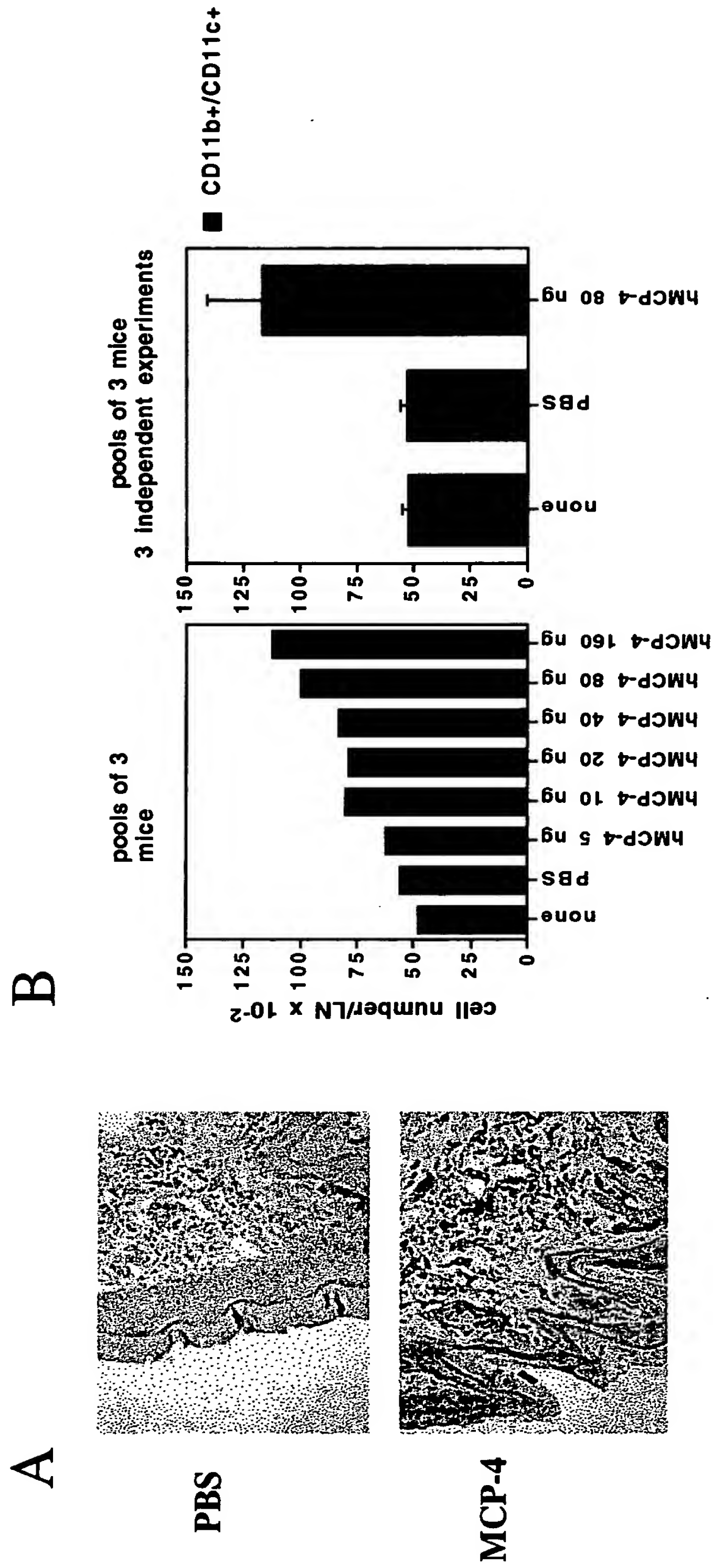
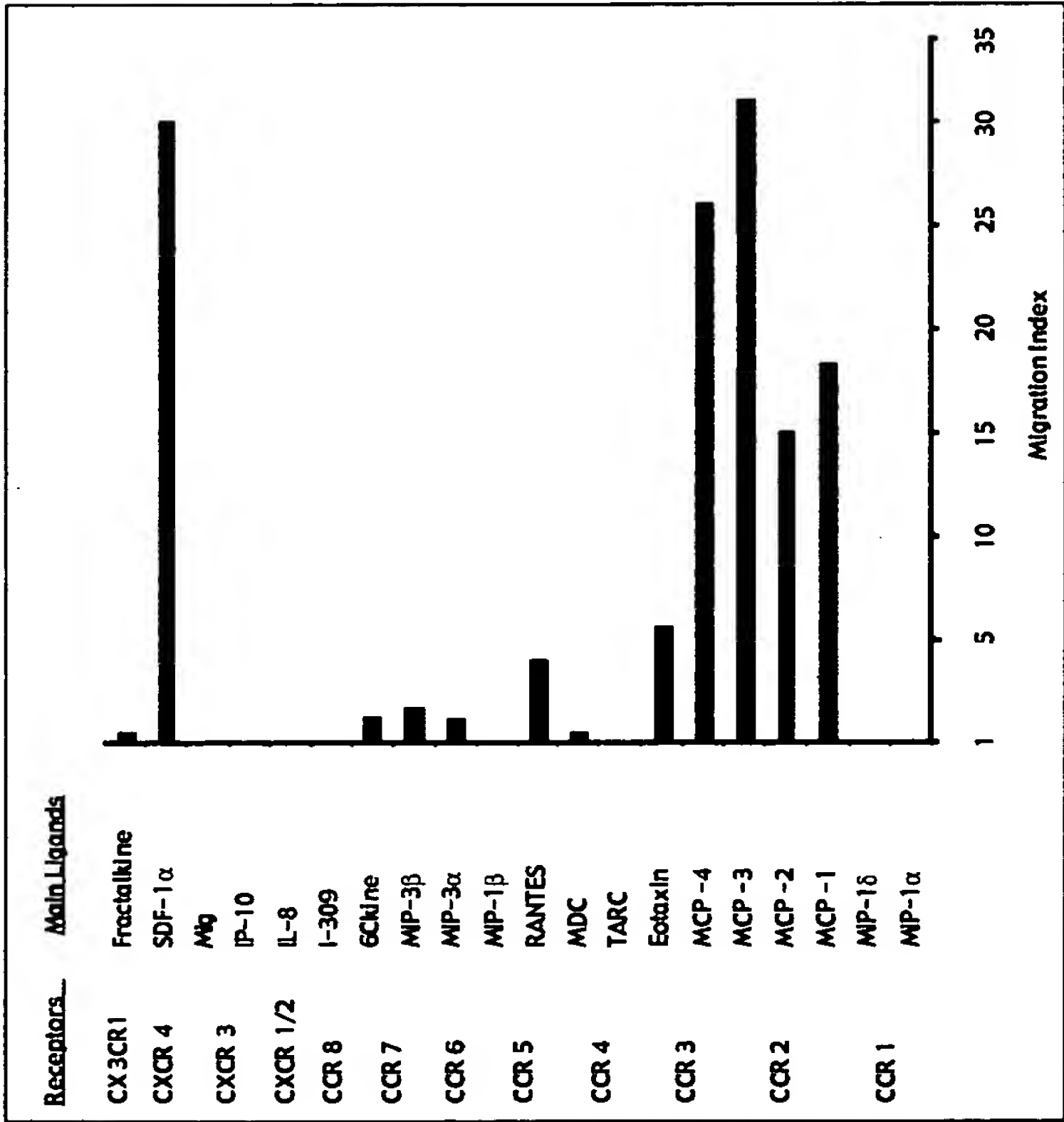


Figure 4

Human MCP-4 is one of the most potent chemokine active on human dendritic cells isolated from blood



Human MCP-4 is active on blood dendritic cells and monocyte-derived dendritic cells, unlike hMCP-1

MCP-4 responsiveness

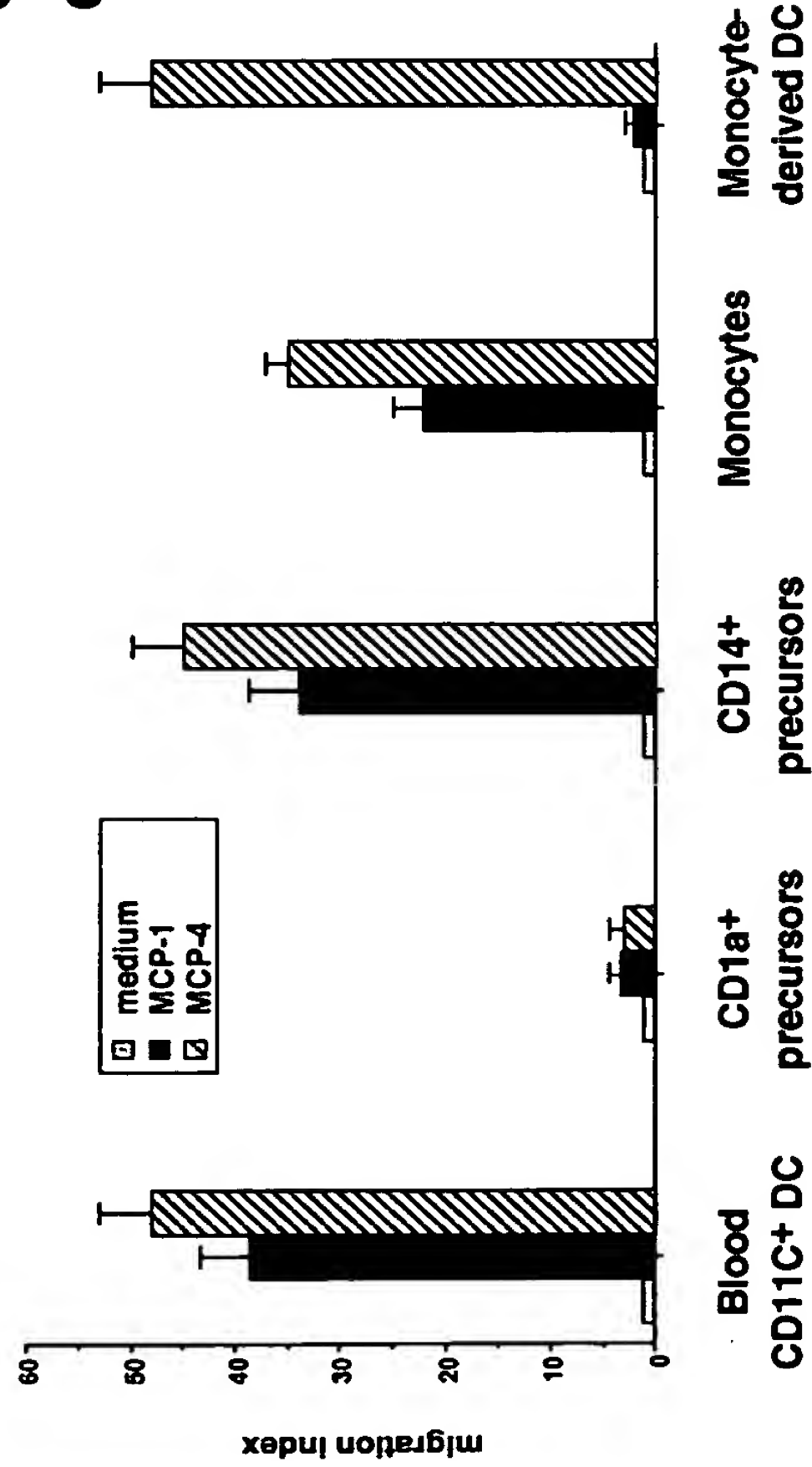
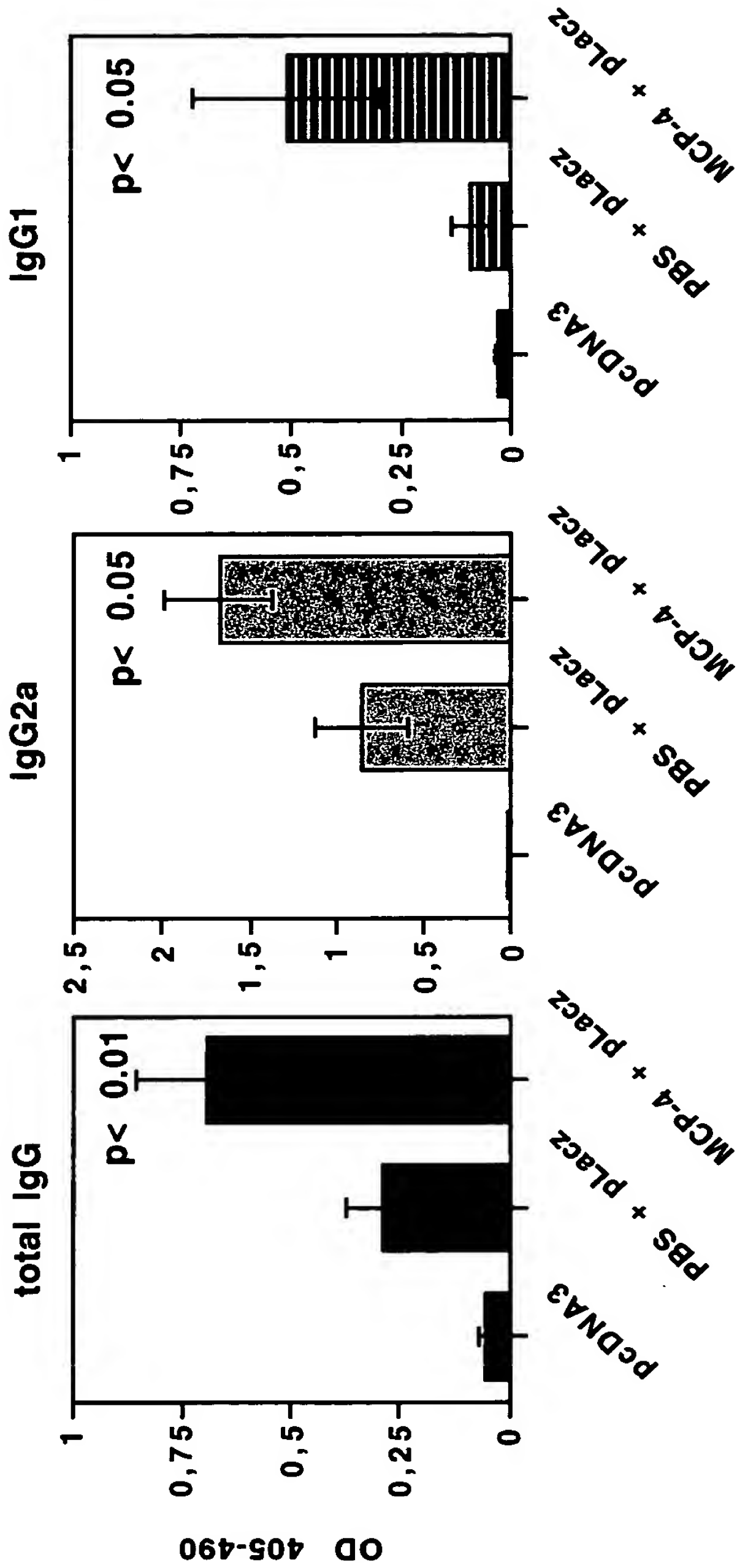


Figure 5

**MCP-4 injection increases the antigen-specific humoral response following beta-galactosidase DNA immunization (50 micrograms DNA injection 3 hours after 100 ng hMCP-4 injection in rear right footpad)**

**Figure shows anti-beta galactosidase antibodies measured after 4 immunizations significance hMCP-4 + pLacZ compared with PBS + pLacZ : Student's t test**



**Figure 6**

MCP-4 injection increases the anti-tumor effect induced by beta-galactosidase DNA immunization (50 micrograms DNA injection 3 hours after 100 ng hMCP-4 injection in rear right footpad, four immunizations prior to tumor challenge) when mice are challenged with a C26 colon carcinoma cell line that expresses beta-galactosidase significance hMCP-4 + pLacZ compared with PBS + pLacZ :  $p < 0.05$  logrank MCP-4 opp: hMCP-4 injected at distant site

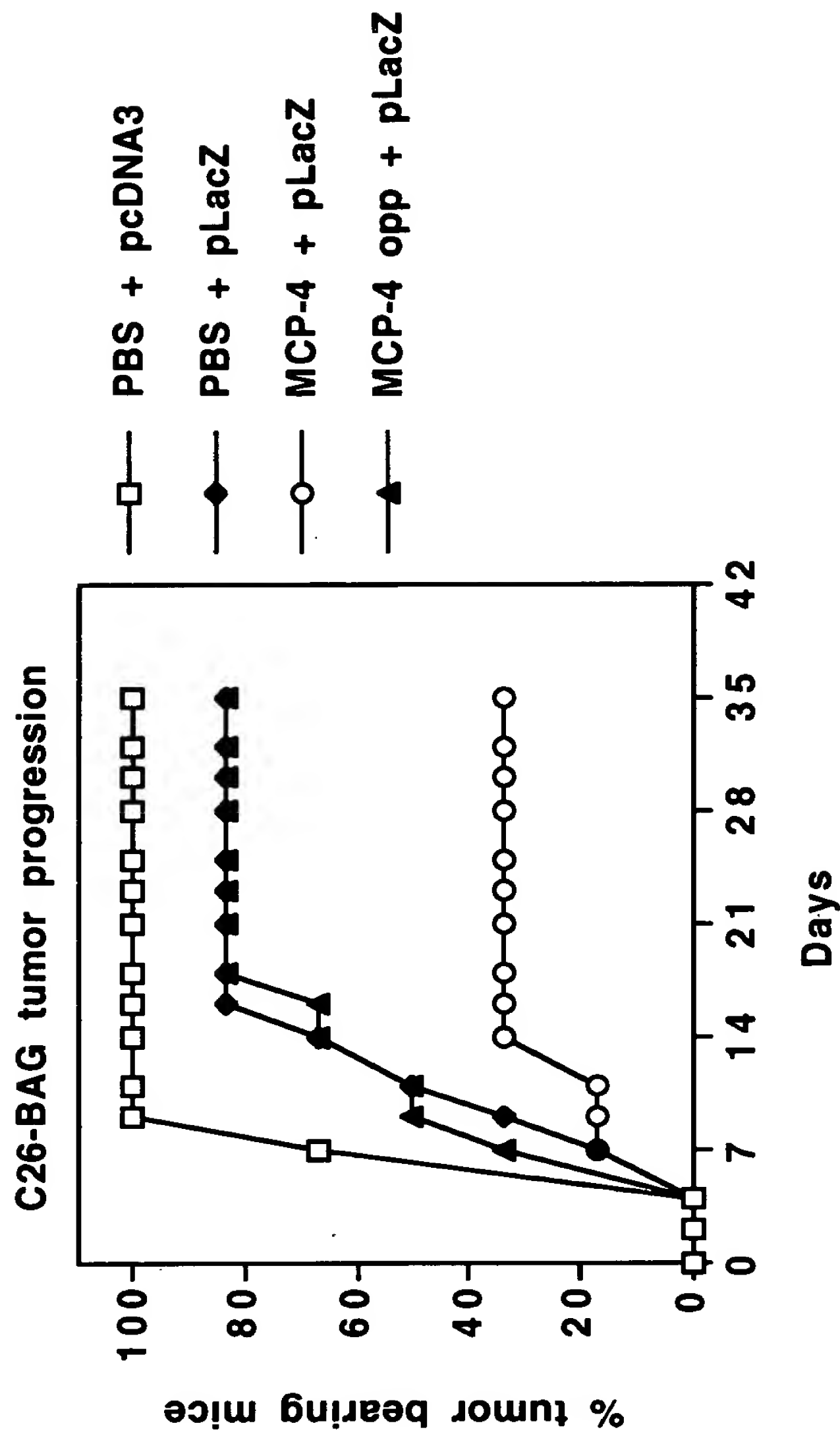
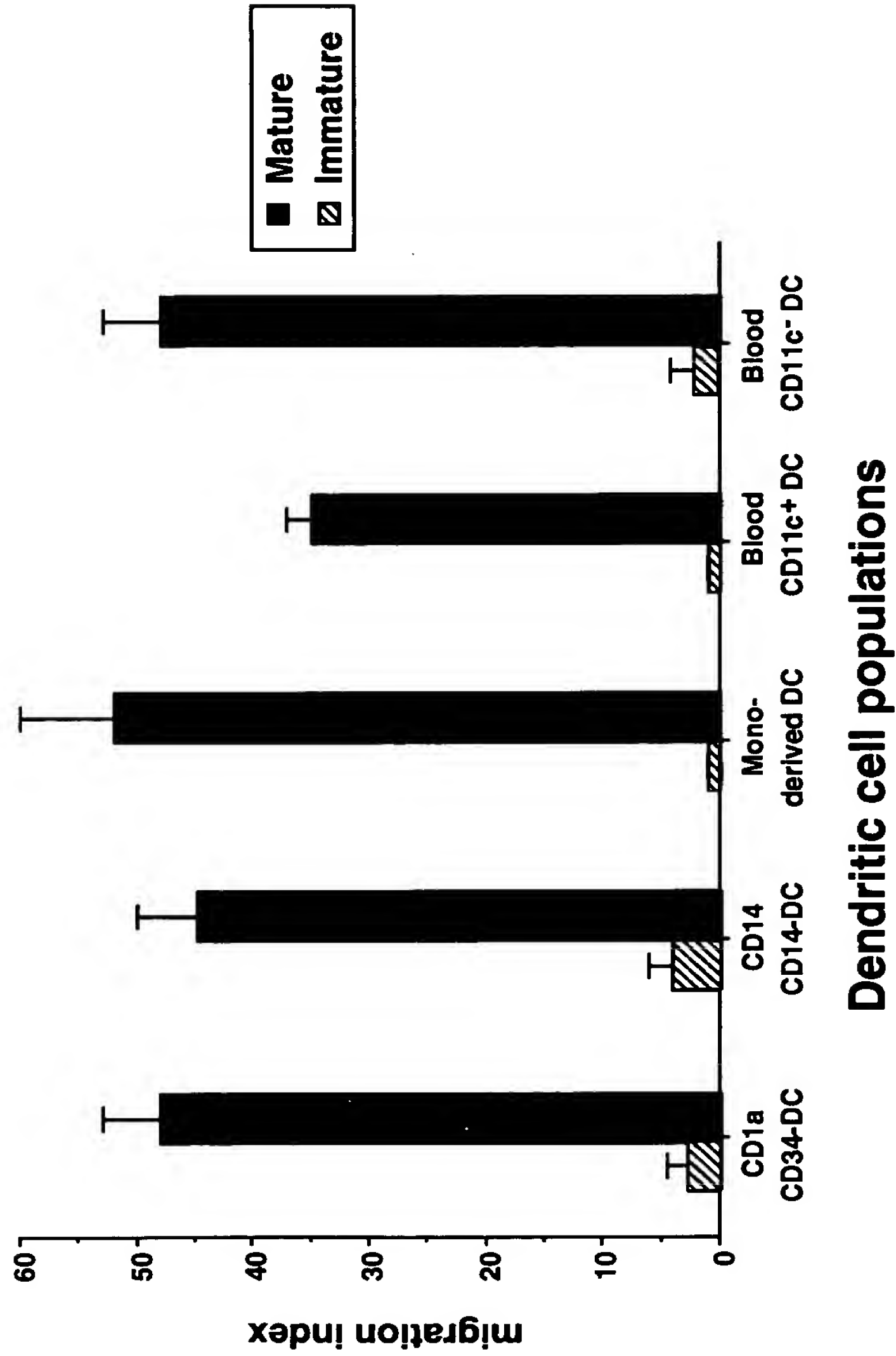


Figure 7

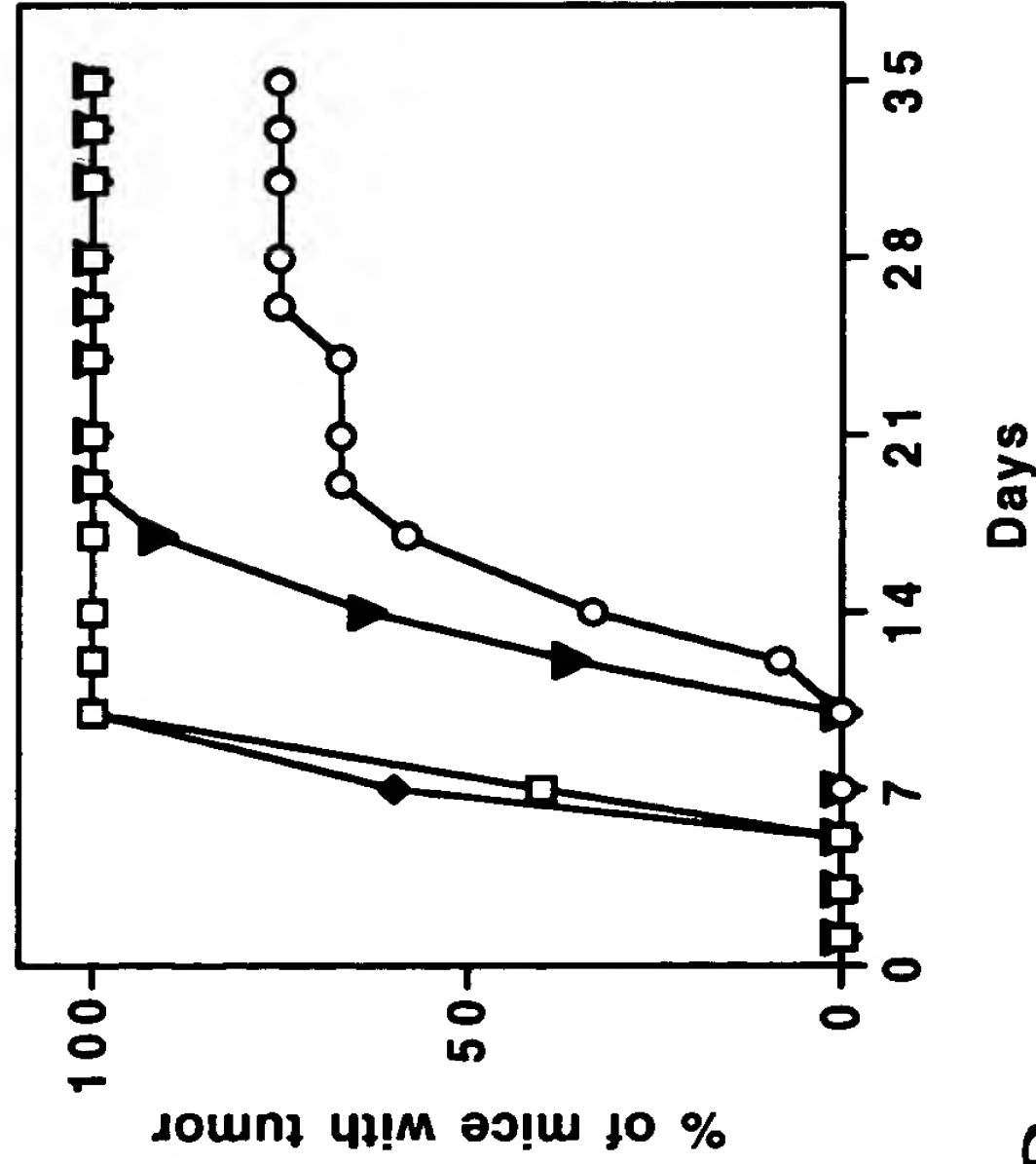


Human 6Ckine is a chemotactic factor for all subsets of human dendritic cells, derived in vitro or isolated ex vivo.

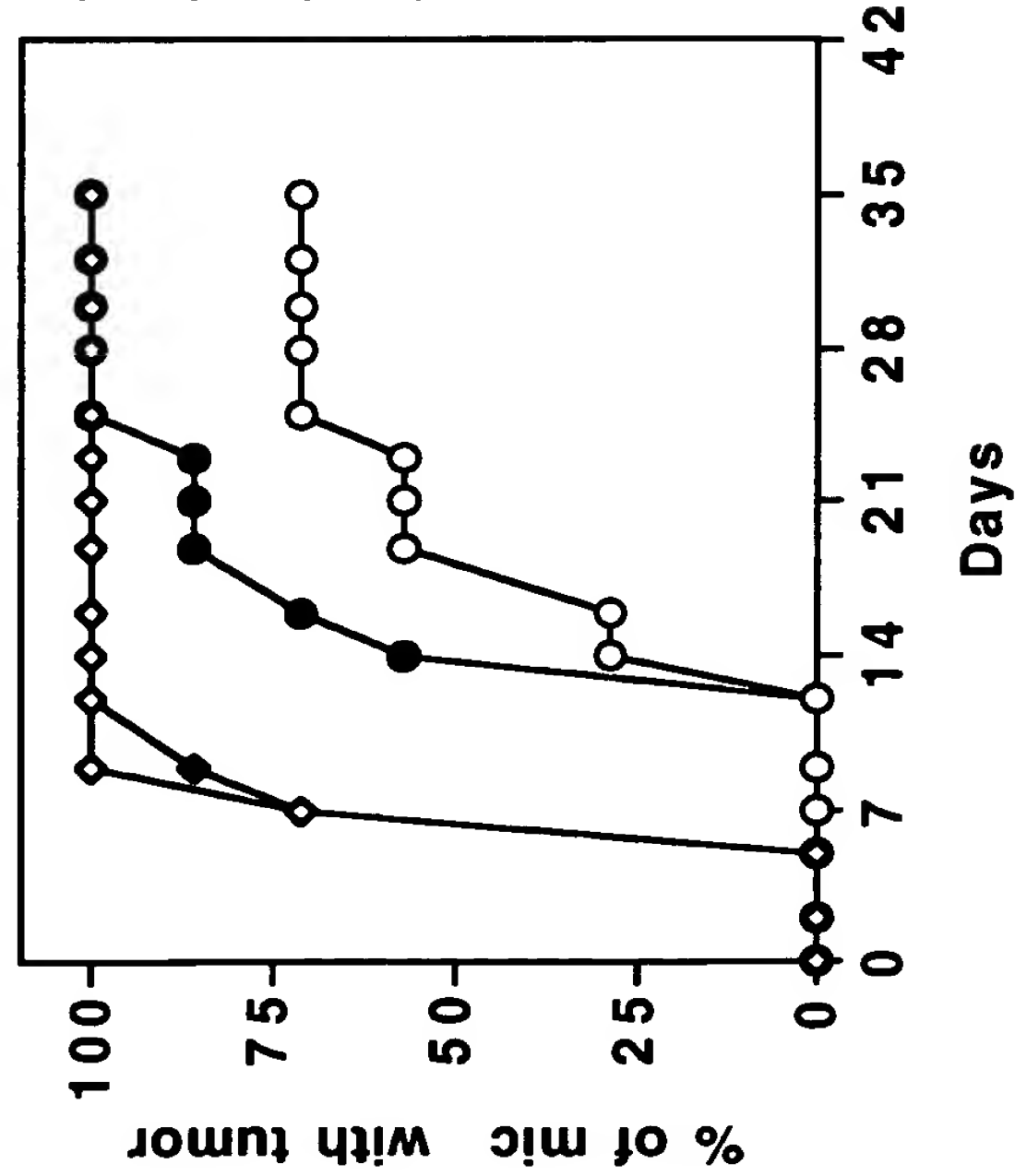
Figure 8



A

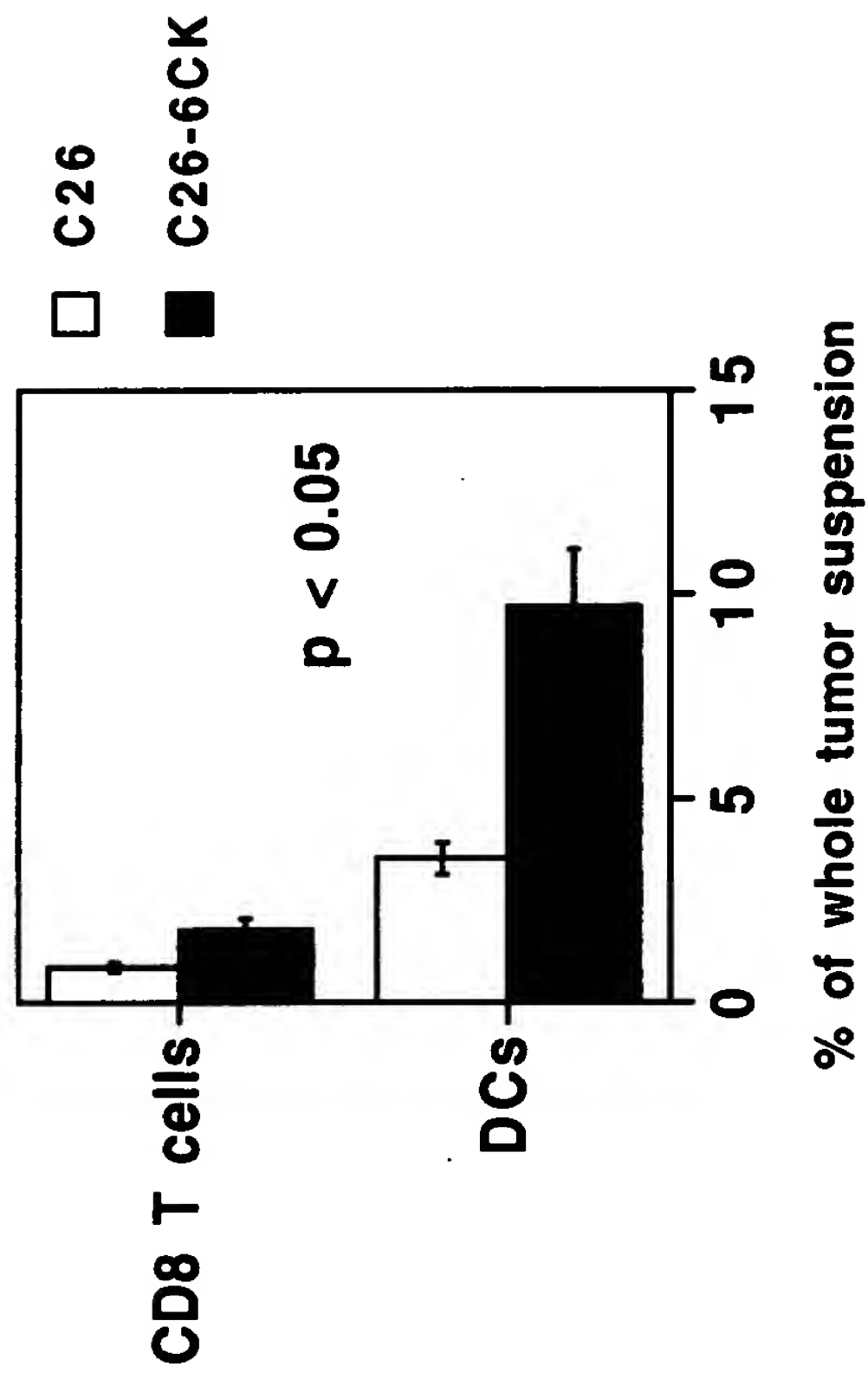


B



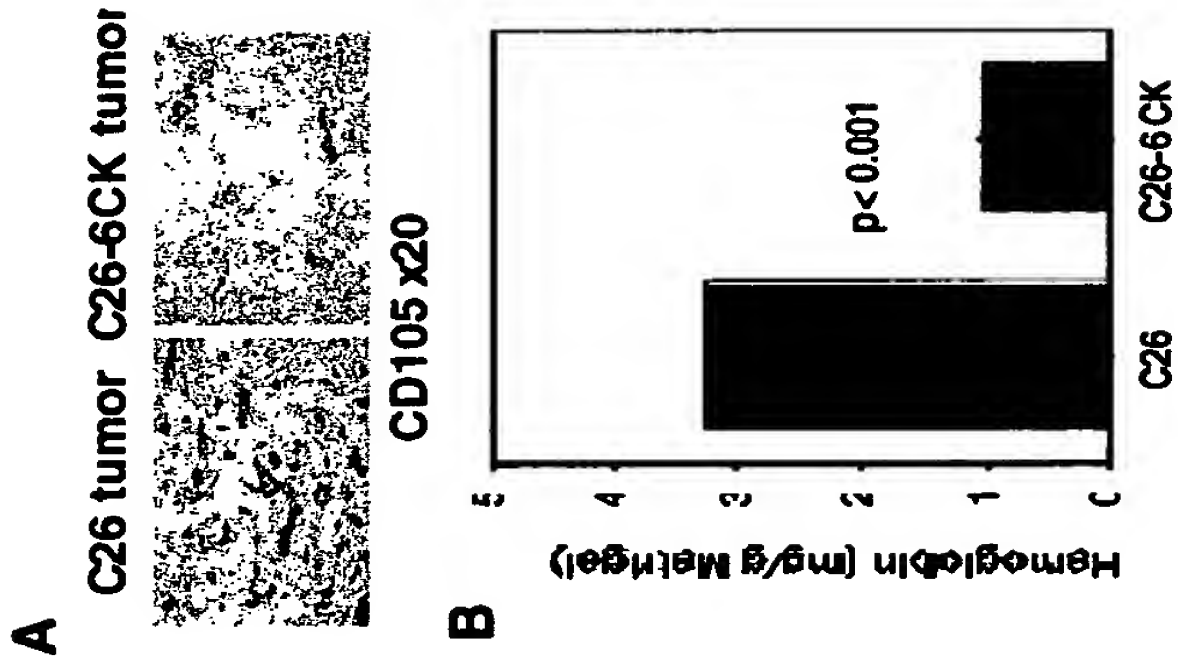
subcutaneous C26-6CK cell injection results in significantly delayed tumor intake compared to parental tumor cells ( $p < 0.01$ ) by logrank analysis (A) and B: C26 + control vs C26-6CK + control). Depleting CD8+ cells (A) or Natural Killer cell activity (B) with specific antibodies in vivo partially reverts the delayed tumorigenicity of the C26-6CK tumor cells, indicating that CD8+ cells and NK cells play a role in delaying tumor growth.

Figure 9



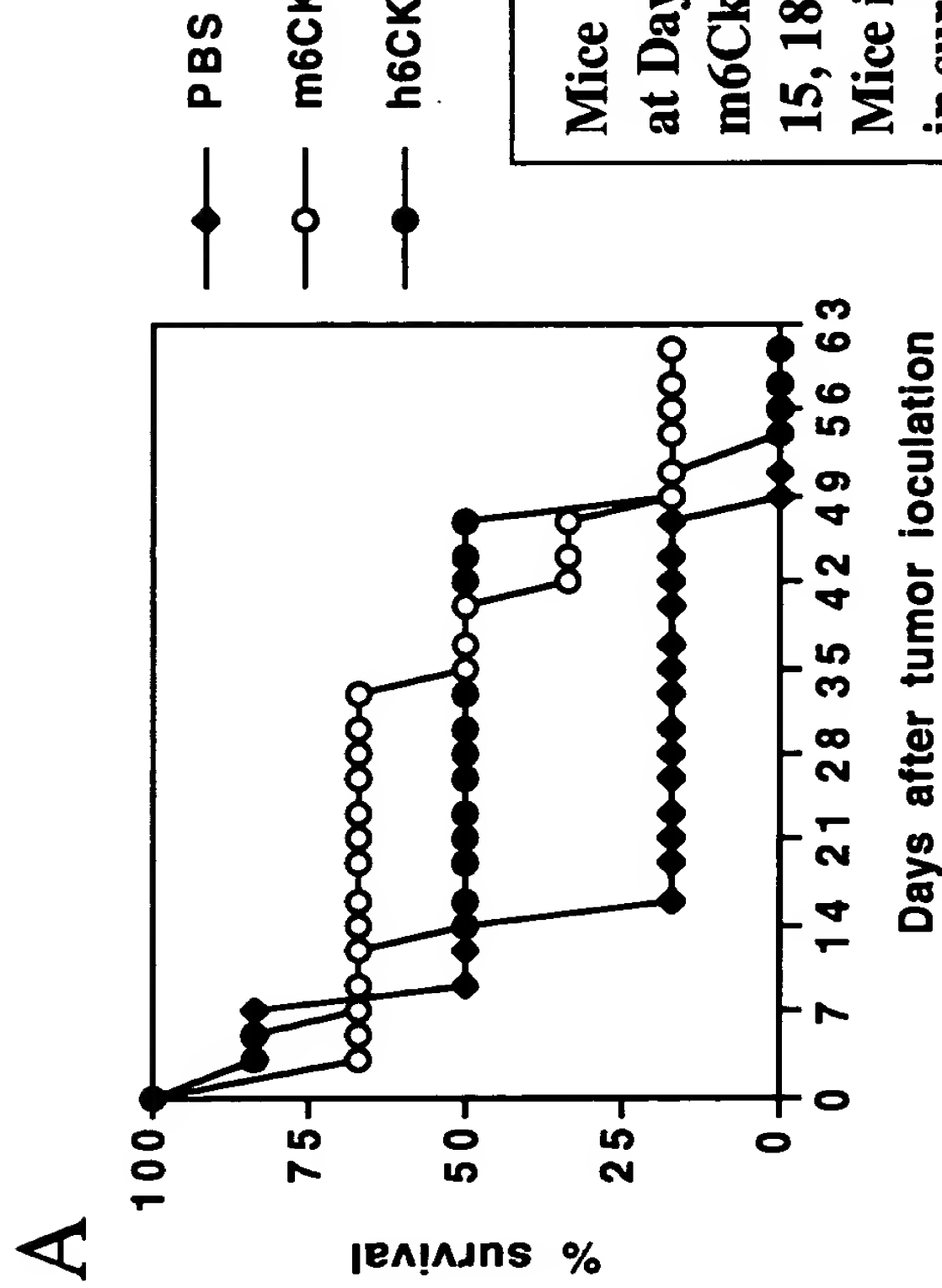
C26 wild-type tumors or C26-6CK tumors expressing m6Ckine have been analyzed for CD8 T cells and CD11c+MHC classII+ dendritic cell (DC) infiltration by flow cytometry analysis of whole tumor suspension (n=7). Data show a significant recruitment of both leukocyte subsets in C26-6CK tumors compared to C26 tumors (Student's t test).

Figure 10



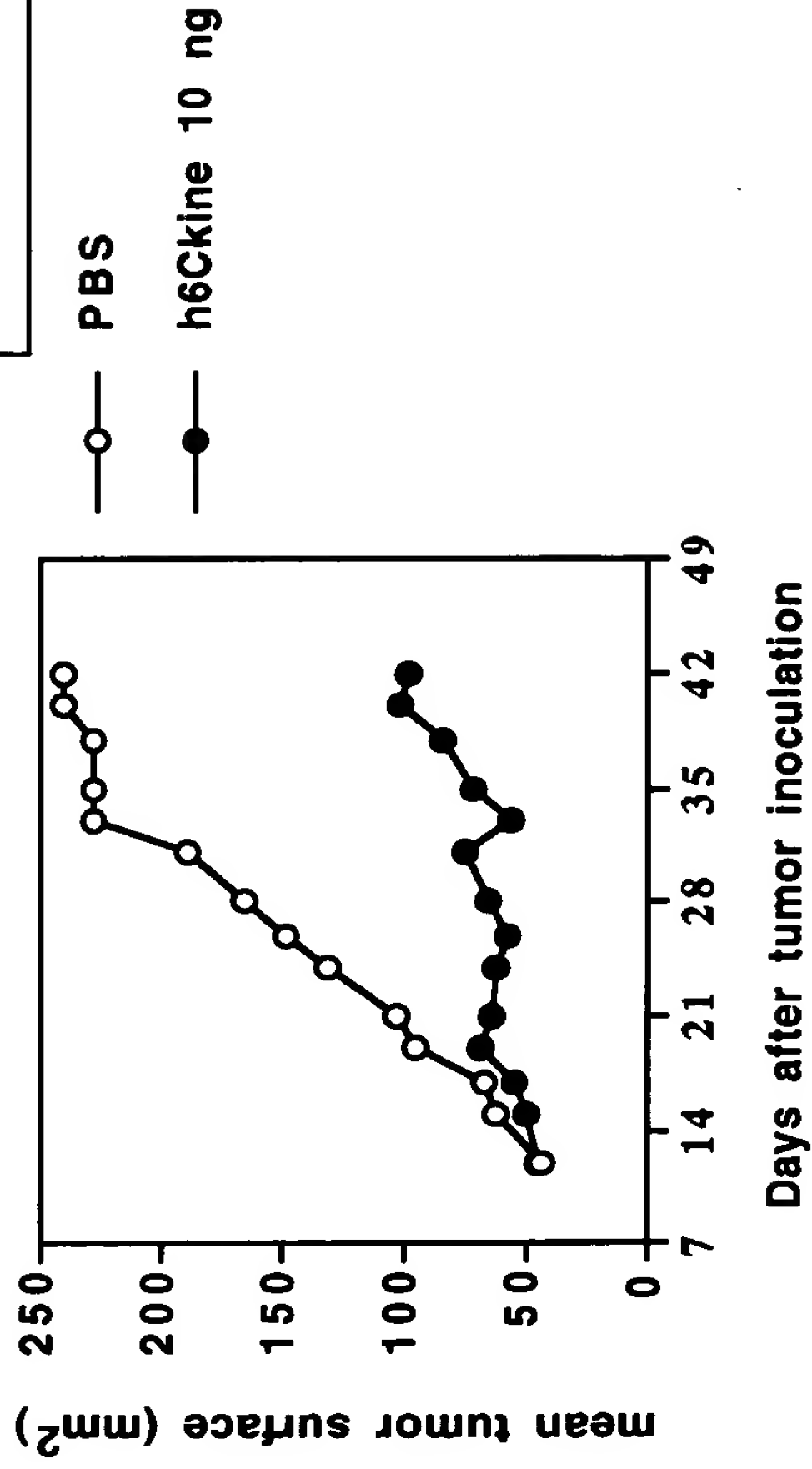
C26 wild-type tumors or C26-6CK tumors expressing m6Ckine have been analyzed for the development of blood vasculature (CD105 staining, A) or angiogenic potential in a Matrigel assay (B). Data show a significant decrease of angiogenesis induced by m6Ckine gene transfer into the C26 tumor.

Figure 11



treatment: D12-D37

**B**



Mice (n=6 / group) were inoculated s.c. with C26 tumor cells at Day 0, randomized and treated with 10 ng recombinant m6Ckine or h6Ckine or PBS intra-tumor at Days 12, 13, 14, 15, 18, 21, 23, 25, 28, 30, 32, 35, 37. Mice injected with h6Ckine and m6Ckine show improvement in survival compared with PBS vehicle alone (A). Injection of h6Ckine also decreased the growth of tumors (B). Note: data on tumor surface obtained from surviving mice.

**Figure 12**

Figure 13

# rAd-m6Ckine treatment of established 4T1 tumors in BALB/c mice

